4.

Latest Developments in Laboratory Haematology: The SYSMEX XT-2000i

Wim van der Meer

Introduction

The introduction of the SYSMEX XE-2100 some years ago demonstrated ingenious adaptation of fluorescence flow cytometry to the field of haematology. The new XT series (XT-2000i and XT-1800i) has inherited these technologies being built inter alia on the same fluorescence flow cytometry (leukocyte differential, immature granulocytes, reticulocytes and optical platelet count), direct current (DC) sheathed flow (red cells, haematocrit, impedance platelets) and sodium lauryl sulphate [SLS] (cyanide-free haemoglobin) platforms, but without counting nucleated red blood cells (NRBC) and without the Immature Myeloid information (IMI) channel. The XT-2000i is illustrated in **figure 1.**

Figure 1
The SYSMEX XT-2000i analyser.

The analyser has a maximum throughput of 80 samples per hour. For closed sample operation, the instrument requires 150 µL of blood. For open sample and capillary sample modes, the figures are 85 µL and 40 µL respectively. The following parameter selections may be made: complete blood count (CBC), CBC + differential, CBC + differential + reticulocytes, CBC + reticulocytes.

This presentation describes an evaluation of the SYSMEX XT-2000i automated haematology analyser performed in the University Medical Centre St Radboud, Nijmegen, The Netherlands. The instrument evaluated was a production model initially adjusted and checked by the manufacturer's representatives. The SYSMEX XE-2100 [**1**] was used as the comparison instrument. Detail of the materials and methods used are given in a recent publication [**2**].

XT-2000i Technology

The optical system of the XT-2000i employs [**1**] a very stable red diode laser producing a light beam of 633 nm wavelength (**figure 2**) and [**2**] a polymethine based fluorescent dye. When the laser beam collides with a stained cell, three signals

are produced: forward scattered light (FSc) providing information on cell size; side scattered light (SSc) providing information on internal cell structure; and finally side fluorescence (Fl) providing information on RNA/DNA content (**figure 3**). The light scatter signals are detected by photo diodes and the fluorescence signal by a photomultiplier via a dichroic mirror.

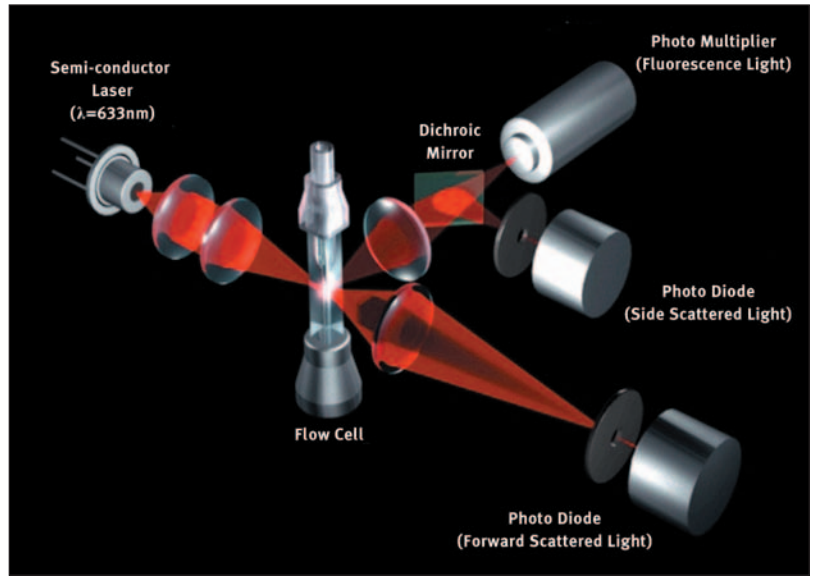

Figure 2
The SYSMEX XT-2000i optical system.

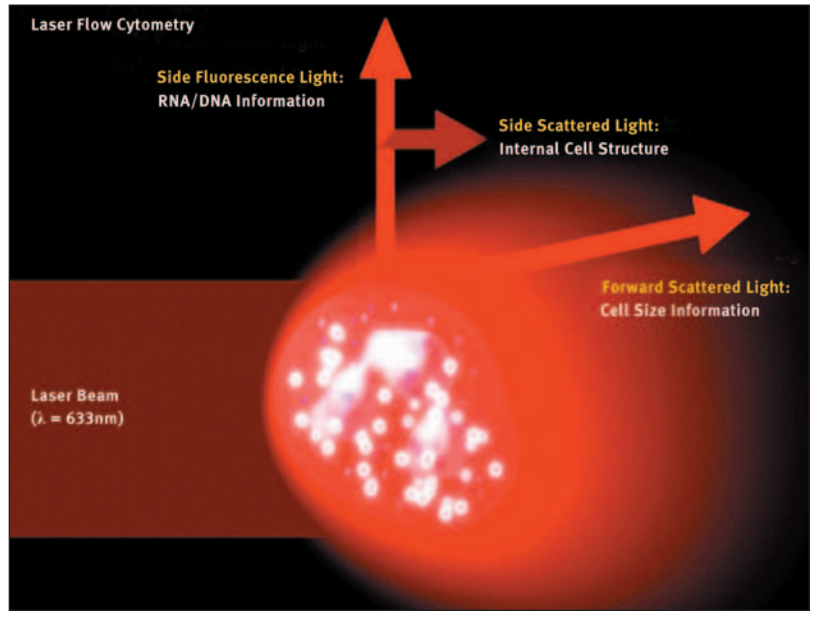

Figure 3
Flow cytometry signals produced by the XT-2000i.

In the WBC/BASO scattergram (**figure 4**) FSc (y-axis) and SSc (x-axis) are employed. RBCs are lysed and a ghost population formed. Basophils are resistant to the reagent system and retain their size and shape forming a cluster of larger cells distinct from other non-basophil nucleated cells whose membranes are perforated and cytoplasm lost. In the DIFF scattergram (**figure 5**) fluorescence (Fl) is on the y-axis and SSc on the x-axis. In the healthy individual, cluster analysis reveals ghost, lymphocyte, monocyte, neutrophil + basophil, and eosinophil populations. The RET (reticulocyte) scattergram (**figure 6**) shows FSc on the y-axis and Fl on the x-axis. The low Fl signal of mature RBCs can be distinguished from the maturing fractions (LFR, MFR, HFR) of the reticulocytes. Platelets are also detected in this scattergram clearly separated from the erythroid cells. Using a logarithmic scale, an enlarged platelet cluster is visible in the PLT-O scattergram (**figure 7**). This is the basis of the fluorescence (optical) platelet count. A good impression of the size and RNA content of the platelets is obtained. The instrument always performs an impedance platelet count but this does possess certain limitations, e.g. red cell fragments in thrombotic thrombocytopenic purpura (TTP), in thalassaemia where extreme microcytes and RBC fragments are found and in giant platelet syndromes such as Bernard Soulier syndrome, May-Hegglin anomaly or in chronic myeloproliferative syndromes. Under these circumstances where an abnormal cell distribution (platelets or RBC) is present, a flag is generated suggesting the need for a repeat using the fluorescence platelet count (PLT-O).

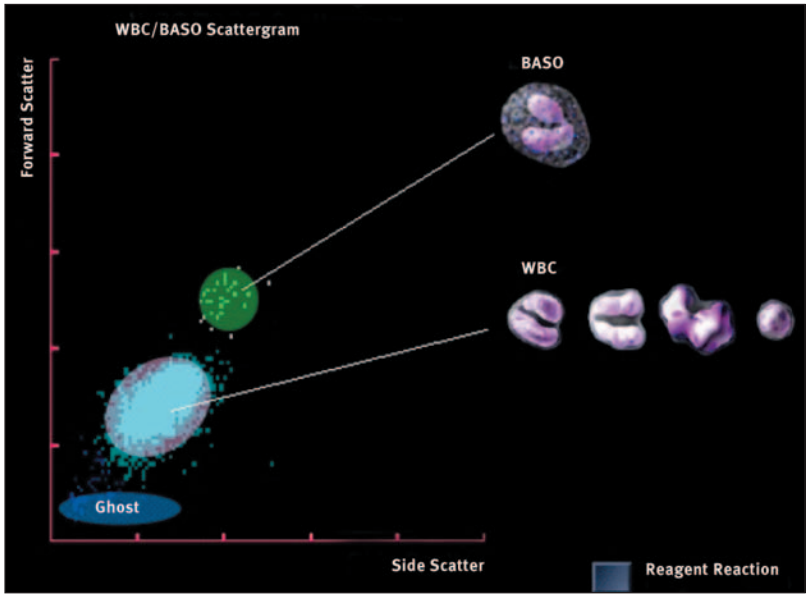

Figure 4
WBC/BASO scattergram from the XT-2000i

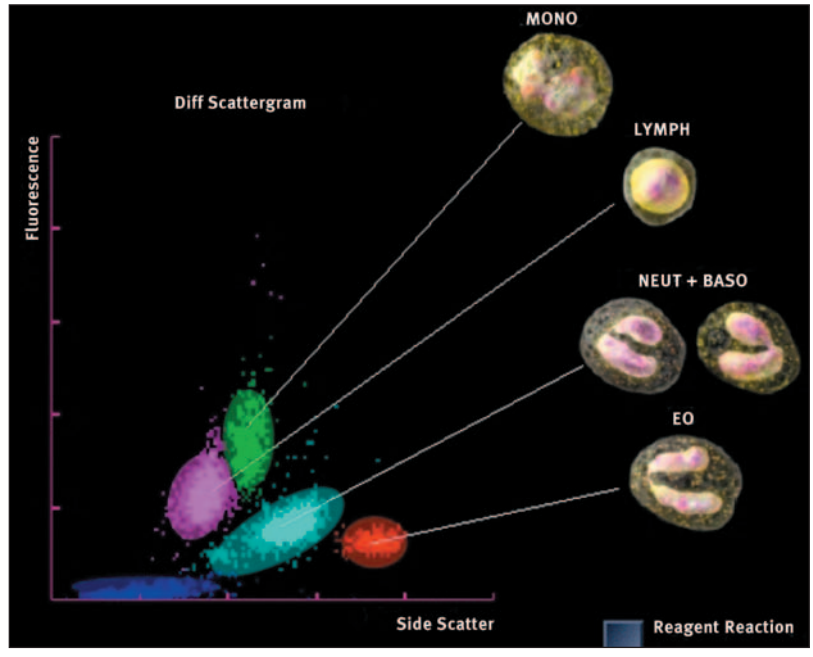

Figure 5
DIFF scattergram from the XT-2000i showing five populations: ghosts (blue), lymphocytes (pink), monocytes (green), neutrophils + basophils (turquoise) and eosinophils (red).

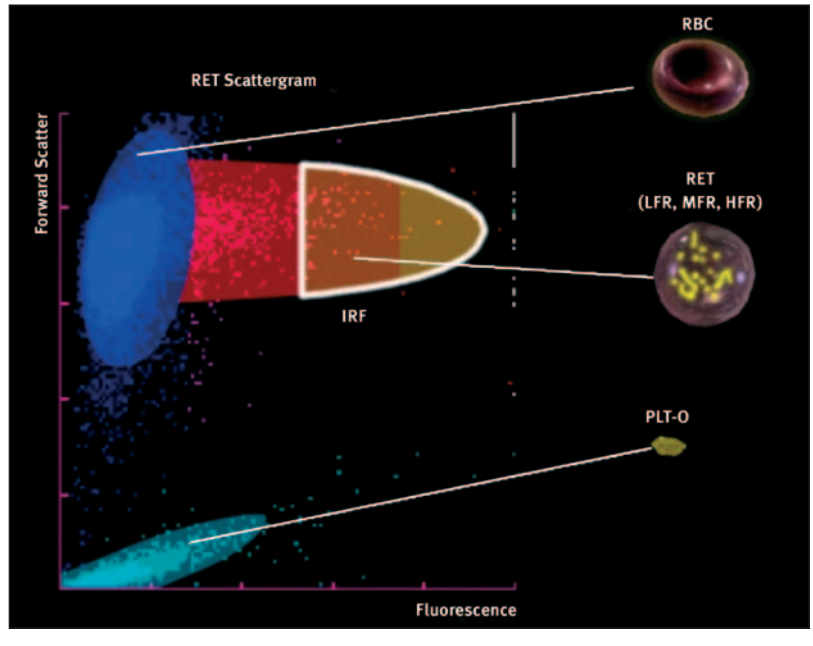

Figure 6
RET (reticulocyte) scattergram.
RBC = red blood cells; LFR, MFR, HFR = low, medium, and high reticulocyte fractions; IFR = immature reticulocyte fraction; PLT-O = fluorescent (optical) platelets

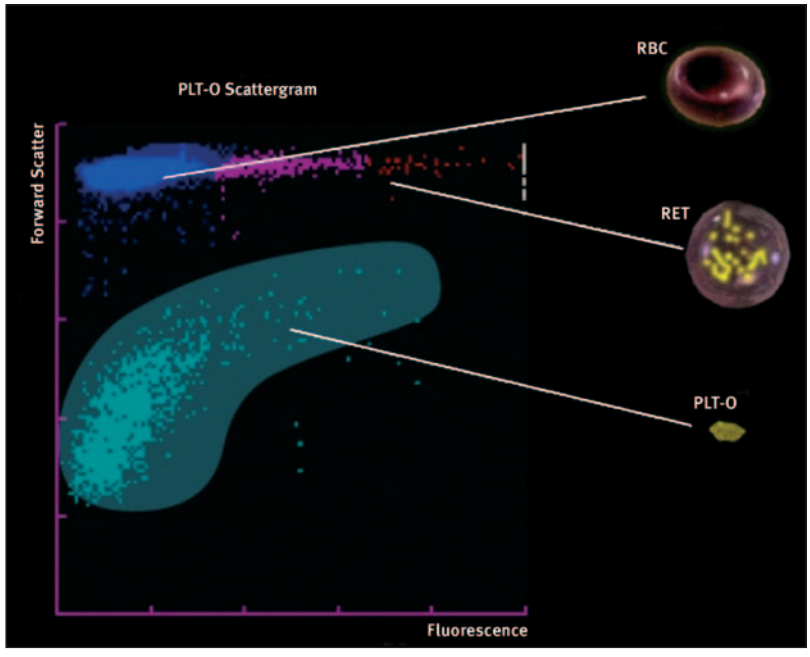

Figure 7
PLT-O scattergram.

Results

Linearity: Linearity studies were performed on multiple dilutions of appropriate specimens to cover the anticipated working range of the analyser. At each dilution, measurements were undertaken both in open and in capillary modes. The results are presented in **table 1**.

Table 1
Linearity studies.
PLT-I = impedance platelet count;
PLT-O = optical platelet count;
RET = reticulocyte count.

Parameter	Open mode range	R^2	Capillary mode range	R^2
WBC	0.8 – 219.0 x 10^9/L	0.999	0.8 – 244.1 x 10^9/L	0.995
RBC	0.38 – 8.27 x 10^{12}/L	0.999	0.35 – 6.86 x 10^{12}/L	0.994
Haemoglobin	0.7 – 15.0 g/dL	0.999	0.7 – 12.8 g/dL	0.994
PLT-I	19 – 1287 x 10^9/L	0.994	13 – 1287 x 10^9/L	0.989
PLT-O	13 – 1669 x 10^9/L	0.989	13 – 1870 x 10^9/L	0.978
RET	16 – 200 x 10^9/L	0.955	29 – 171 x 10^9/L	0.949

Repeatability: For each parameter, specimens of low, normal and high concentrations were measured 10 times. The results are presented in **table 2**. The high CV% for the reticulocytes was due to the low reticulocyte counts, a phenomenon well recognised by statisticians.

Parameter	Count range	CV range (%)
WBC	1.8 – 17.1 x 10^9/L	2.1 – 1.9
RBC	2.44 – 5.84 x 10^{12}/L	0.8 – 0.3
PLT I	19.4 – 1109.7 x 10^9/L	3.6 – 1.3
PLT-O	21.2 – 1247.9 x 10^9/L	3.9 – 1.6
RET	0.2 – 4.0 %	53.0 – 0.3

Table 2
Repeatability study.

Carry-over: This study was undertaken in accordance with the recommendations of the International Council for Standardization in Haematology (ICSH) [**3**].

Parameter	Carry-over
WBC	0.12%
RBC	0.0%
PLT I	0.0%
PLT-O	0.75

Table 3
Carry-over study

Comparability: Two hundred specimens were measured on both the XT-2000i and the XE-2100 (100 healthy individuals and 100 patients). Selected parameter correlations are shown in **table 4**. For the comparisons for all parameters consult reference [**2**] which also includes Bland-Altman plots.

Parameter	Unit	Intercept	Slope	Correlation Coefficient (r)
WBC	x 10^9/L	-0.0216	0.983	0.998
RBC	x 10^{12}/L	-0.1515	1.035	0.998
Haemoglobin	g/dL	-0.100	1.000	0.998
MCHC	g/dL	0.0400	1.000	0.914
RET-HFR	%	0.00	1.016	0.829
PLT-I	x 10^9/L	-1.79	1.013	0.992
PLT-O	x 10^9/L	1.36	0.888	0.996

Table 4
Comparison of XT-2000i and XE-2100 analysers for selected parameters.

Flagging: For assessment of the flagging capability of the XT-2000i, the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [**4**] were followed. Comparison of flags produced by the XT-2000i and manual methods (400-cell differential counts by two technologists each performing a 200 cell count) was made by analysing 100 normal and 100 abnormal samples.

Flag	Specificity (%)	Sensitivity (%)	Efficiency (%)
Abnormal lymphocytes/ blasts? Atypical lymphocytes?	71.0	92.0	89.9
Blasts?	96.6	100.0	96.0
Immature granulocytes	79.7	100.0	81.3
Left shift	95.6	70.4	92.6
NRBC	92.5	81.3	91.7

Table 5
Specificity and sensitivity of XT-2000i analyser flagging.

Specificity, sensitivity and efficiency figures are presented in **table 5**. It is important to note that the flagging capabilities of all automated haematology analysers should not be overestimated. Clinical information is necessary for the correct interpretation of the differential leukocyte count.

Illustrative Cases

During any evaluation a number of interesting and illustrative cases are found which display attributes of analyser performance not revealed by the simple statistics of technical evaluation. Six such cases will now be shown.

Case 1: This DIFF scattergram (**figure 8**) is so abnormal that cluster analysis is not possible but clearly some very abnormal cells are present. The smear shows very immature cells with the characteristics of monoblasts. The diagnosis of acute myelomonoblastic leukaemia was confirmed by immunophenotypic analysis.

Case 2: Again the DIFF scattergram is grossly abnormal (**figure 9**). Although similar to the previous case, the abnormal cluster indicates smaller cells, in this case the blast cells of acute myeloid leukaemia.

Case 3: The DIFF scattergram (**figure 10**) shows a large abnormal population in the monocyte and atypical lymphocyte area. Lymphoblasts or atypical lymphocytes may be anticipated, however, the co-existence of severe anaemia and thrombocytopenia suggest acute lymphoblastic leukaemia, which was, in fact, confirmed.

Case 4: The DIFF scattergram in this case (**figure 11**) is not unlike that in figure 10. However, in the absence of anaemia and thrombocytopenia, the abnormal cluster is more likely to consist of atypical lymphocytes. This, indeed, was the case, the peripheral blood showing the atypical lymphocytes characteristic of infectious mononucleosis.

Case 5: In this case the neutrophil cluster is abnormal (**figure 12**), showing extension into the area characteristic of immature granulocytes. The peripheral blood film shows band cells and immature granulocytes. This patient had severe sepsis.

Case 6: The RET scattergram (**figure 13**) from the final patient shows an abnormal mature RBC population. The forward scatter light signals are lower than usual, indicating a microcytic RBC population. The peripheral blood film revealed the microcytic, hypochromic blood picture of severe iron deficiency.

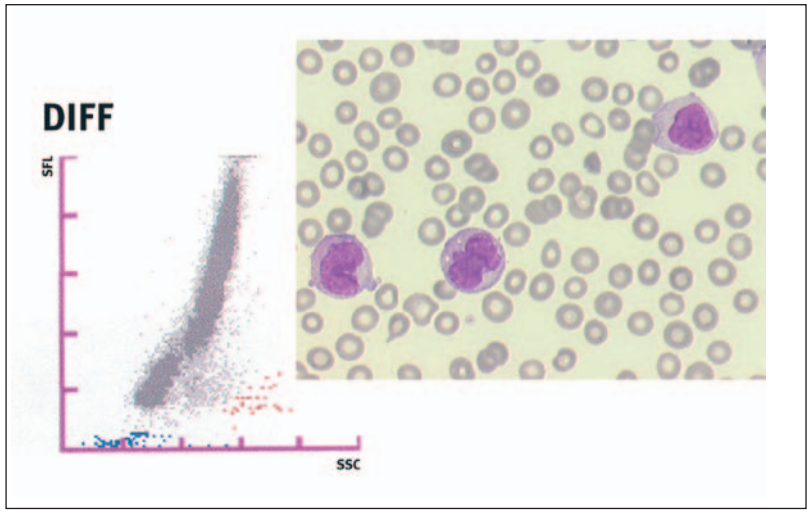

Figure 8
DIFF scattergram from a patient with acute myelomonoblastic leukaemia. Note the primitive monocytoid cells in the peripheral blood.

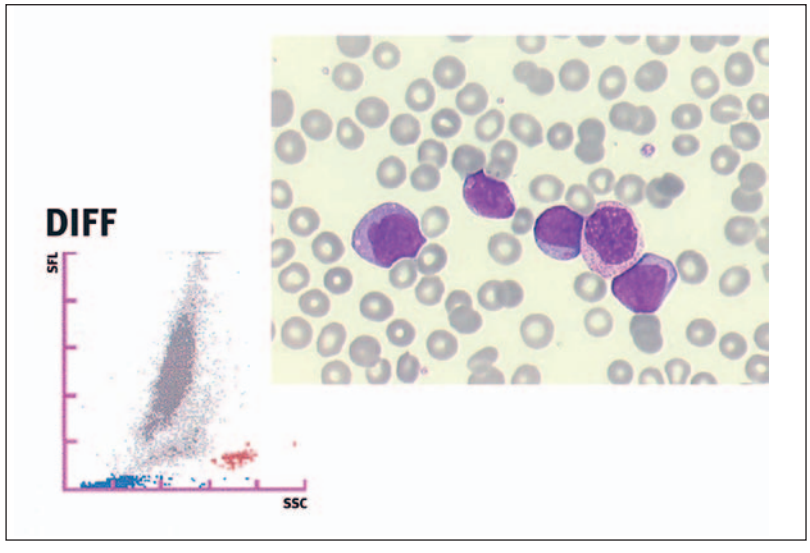

Figure 9
DIFF scattergram from patient with acute myeloid leukaemia.
Note the myeloblasts in the peripheral blood.

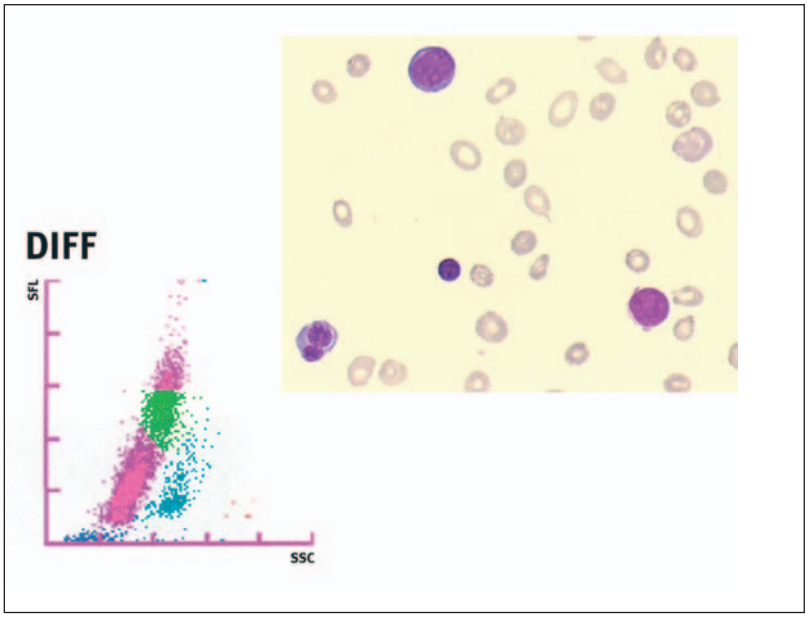

Figure 10
DIFF scattergram from a patient with acute lymphoblastic leukaemia. The appearance is not pathognomonic of ALL but the coexistent severe anaemia and thrombocytopenia make this the most likely diagnosis. Note the lymphoblasts at 5 o'clock and 11 o'clock and the binucleate NRBC at 7 o'clock.

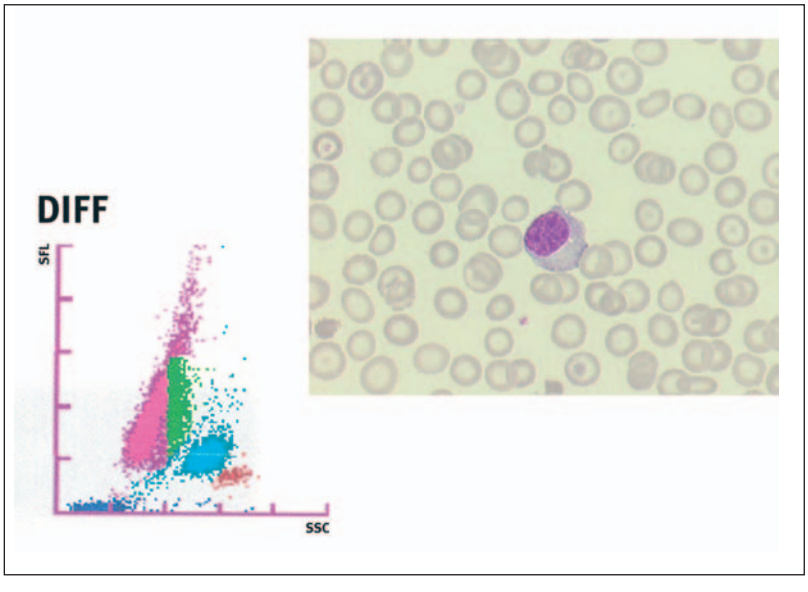

Figure 11
Note the similarity to the DIFF scattergram illustrated in figure 10, but the absence of anaemia and thrombocytopenia makes this less likely to be malignant. This patient has infectious mononucleosis.

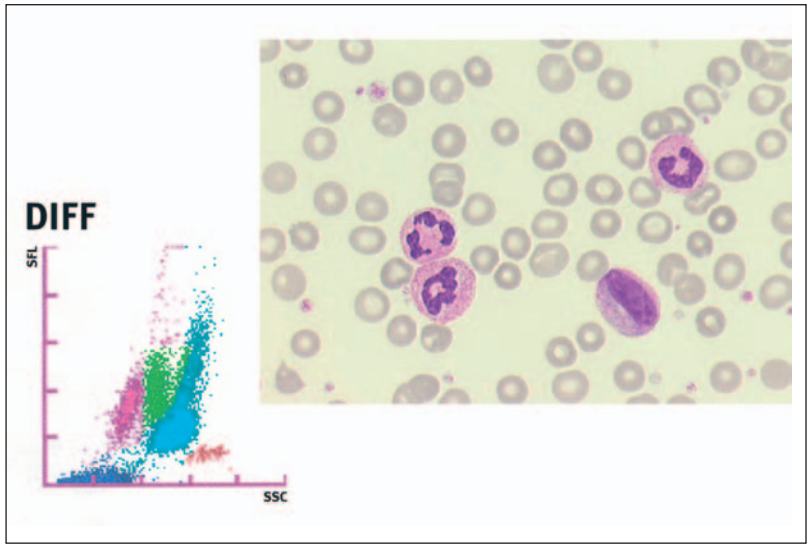

Figure 12
DIFF scattergram from a patient with severe sepsis. Note the expansion of the neutrophil cluster into the immature granulocyte area. Note the band cell and myelocyte.

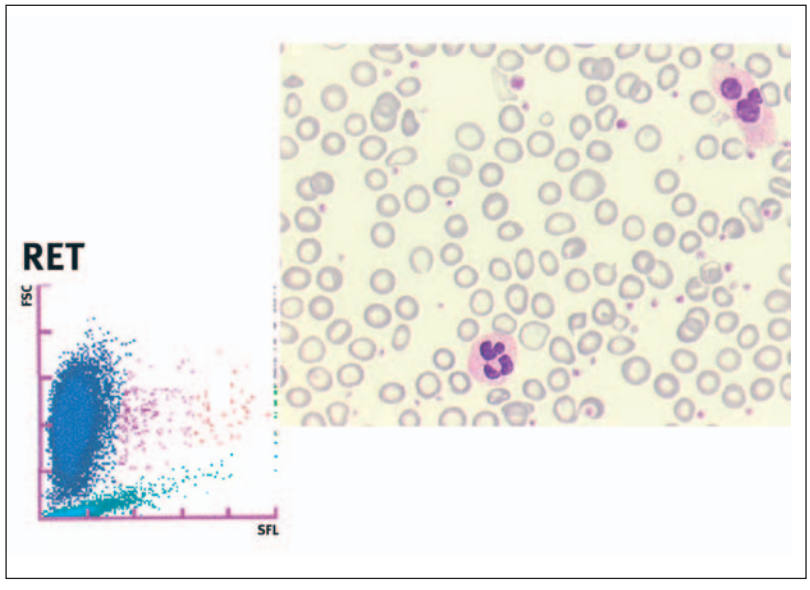

Figure 13
RET (reticulocyte) scattergram. Note the FSc signals are lower than usual indicating microcytic RBC. Note the microcytic hypochromic RBC indicating severe iron deficiency anaemia.

Conclusions

This evaluation has demonstrated that the XT-2000i is a reliable analyser. Satisfactory linearity is established for all parameters over a useful working range; reproducibility is good and carry-over zero or minimal. All XT parameters compare well with those of the XE-2100 and the flagging capacity is also comparable. There is a definite place for this analyser in a laboratory not requiring the augmented output and extremely high throughput of the XE-2100.

Acknowledgements

Successful evaluation of a major haematology analyser requires the collaboration of many individuals, both laboratory and clinical. I should like to take this opportunity to thank colleagues in the Departments of Clinical Chemistry and Special Haematology for their assistance throughout this evaluation. In particular I should like to express my thanks to Ries de Keijzer and Jacqueline Dinnissen. I would also like to thank the staff of SYSMEX Europe and Goffin Meyvis for the opportunity to evaluate the XT-2000i.

References

[1] **Briggs C., Harrison P., Grant D.,** *et al* (2000)
New quantitative parameters on a recently introduced automated blood cell counter – the XE-2100™. Clin Lab Haemat 22, 345–350

[2] **van der Meer W., Dinnissen J.M.B., de Keijzer M.H.** (2002)
Evaluation of the SYSMEX XT-2000i, a New Automated Haematology Analyser. SYSMEX J Int 12,71–75

[3] **ICSH** (1994)
Guidelines for evaluation of automated blood cell analysers including those used for differential leukocyte and reticulocyte counting and cell marker applications. Clin Lab Haemat 16, 157–174

[4] **NCCLS** (1992)
Reference Leukocyte Differential Count (Proportional) and Evaluation of Instrumental Methods: Approved Standard NCCLS Document H20-A.